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Analysis of Physicochemical and Microbiological Parameters of Wine Produced from Banana and Pineapple

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ABSTRACT

Wine is a fermented drink made by the controlled culture of yeasts on fruit juices. This study was undertaken to produce acceptable wines from blends of banana and pineapple by the fermentative action of *Meyerozyma guilliermondii* strain 1621 and *Pichia guilliermondii* strain PAX-PAT 18S. The fermentation process lasted for a period of 28 days and, the aging process was for 2 months. The fermentation process comprised two set ups- one was fermented by *Meyerozyma guilliermondii* strain 1621 and the other was fermented by *Pichia guilliermondii* strain PAX-PAT 18S. The process was monitored and controlled by carrying out physicochemical analysis (pH, temperature, specific gravity, total titratable acidity, and alcohol content) and yeast count using standard methods. There was a decrease in the pH for both wines and an increase in the total titratable acidity. The temperature was between 17 and 27 ^{0}C for both wines. The specific gravity of the wines decreased during the fermentation leading to an increase in alcohol production. There was an increase in yeast count from 6.7×10^7 sfu/ml to 1.8×10^8 sfu/ml between days 1 and 17 and a decrease from 1.8×10^8 sfu/ml to 0 sfu/ml between days 17 to 85 for *Meyerozyma guilliermondii;* also an increase from 5.1×10^7 sfu/ml to 1.7×10^8 sfu/ml from day 1 to 17, and a decrease from 1.7×10^8 sfu/ml to 0 sfu/ml between day 17 to 85 for *Pichia guilliermondii*. Statistically, there was no significant difference between the yeast counts, temperature, pH, total titratable acidity, and specific gravity but there was signa ificant difference between the alcohol production for both wines. This study shows that wines can be successfully produced using *Meyerozyma guilliermondii* strain 1621 and *Pichia guilliermondii* strain PAX-PAT 18S.

Keywords: Fermentation; Wine production; Banana and pineapple substrates; *Meyerozyma guilliermondii; Pichia guilliermondii*; Physicochemical analysis; Microbiological analysis.

1.0. Introduction

Wine is an alcoholic beverage made from fermented grapes or other fruits and plants. Fruit wines are undistilled alcoholic beverages that have undergone a period of fermentation and aging. They are nutritive, tasty, and act as mild stimulants [1]. Wine production has been practiced with various fruits such as apples, pears, strawberries, cherries, plums, bananas, pineapples, oranges, cucumbers, watermelon, guava etc. using species of *Saccharomyces cerevisiae* which converts the sugar in the fruit juices into alcohol and organic acids, that later react to form aldehydes, esters and other chemical compounds which also help to preserve the wine [2,3]. The wine so produced bears the name of the fruit or fruit mixture used in its production. For the past few decades, grapes have been the main fruits used for wine production.

In Nigeria, there is an abundance of tropical fruits which include paw-paw, watermelon, pineapple, orange, plum, etc. These fruits are difficult to keep for a considerable length of time, hence the ripe fruits are utilized either as fresh or processed into juice and other products [4]. High wastage of these fruits especially at their peak season of production necessitates the need for alternative preservation towards an enhanced utilization of these fruits. The production of wines from common fruits could thus, help reduce the level of post-harvest loss and increase the variety of wines [5, 6].

Banana (*Musa acuminata*) serves as a good nutritional source of carbohydrates, minerals (such as potassium), and vitamins (such as B1, B2, B3, B12, C, and E). Following the high nutritional content of banana, it is consumed in

large quantity all over the world. The banana fruit can be eaten raw, cooked, processed into flour, or fermented for the production of beverages such as banana juice, beer, vinegar, and wine [7-9]. Also, fermenting banana juice into wine is considered to be an attractive means of utilizing surplus bananas, since the consumption of banana wine provides a rich source of vitamins and ensures the harnessing of the fruits into a useful by-product [10]. Pineapple (*Ananas comosus*) contains vitamins and minerals including potassium, copper, manganese, calcium, magnesium, vitamin C, thiamin, pyridoxine, folate as well as soluble and insoluble fiber and bromelain [11]. The high sugar content in pineapple also makes it a suitable substrate for wine production [12].

Studies have reported *Pichia fermentans* and *P. guilliermondii* as good strains for "must" fermentation as a mixture with *Saccharomyces cerevisiae* improves the aroma as well as the characteristic features of the wine [13 and 14]. *Meyerozyma guilliermondii* is often used for wine colour improvement because of its high hydroxyl cinnamate decarboxylase enzymatic activity [14].

Fermentation is a metabolic process that enhances the release of energy from sugar or other organic molecules, which do not require oxygen or an electron transport system, and uses an organic molecule as the final electron acceptor [15]. During fermentation, it is important to monitor and control the process because factors such as pH, temperature, sugar content, acid, and microorganisms can affect the overall outcome of the wine.

pH directly affects the stability of wine. This is because, at a pH of 7.0 (neutral), most microorganisms (like bacteria, moulds, and some yeasts) become more active for fermentation and subsequent spoilage of wine. pH below 3.5 eliminates most of these microorganisms and favours only a few for fermentation [16]. Similarly, the temperature should not exceed 29.4 ^{0}C for red wines and 15.3^{0}C for white wines, otherwise, this will stop the growth of the yeast cells. A lower temperature is desirable because it increases the production of esters, aromatic compounds, and the alcohol itself. This makes clearing of the wine easier and the wine is less susceptible to bacterial infection [17].

Sugar is the main substrate for the fermentation of fruits into alcohol [18]. Although food nutrients such as fats and proteins can be broken down where sugar is limited, as long as there is the availability of sugar, yeast cells will continue the process of fermentation until other factors become unfavourable [19]. Acid has a direct effect on the quality of the wine but owes its composition to citric, tartaric, and some traces of lactic acid which replaces malic acid during malolactic fermentation, and these acids in wine can be determined by titration [20]. Wines produced from grapes in cold climates tend to have a high concentration of malic acid, a lower pH of between 3.0- 3.5, and the taste slightly decreases in acidity. Decreasing the acidity also increases the pH values which can allow spoilage organisms to grow and carry out malolactic fermentation [21]. In this study, banana and pineapple fruit pulp were used for the production of wine using *Meyerozyma guilliermondii* and *Pichia guilliermondii*, and the physicochemical and microbiological evaluation of the wine was also carried out.

2.0. Materials and Methods

2.1. Sample collection

Ripe Queen pineapple (*Ananas comosus*) and Cavendish banana (*Musa acuminata*) fruits were purchased from Mile 3 market, Port Harcourt, Nigeria, and used to prepare the "must" using the method described by Ogodo *et al.*

[22]. The "must" was in the ratio of 3:1 for pineapple and banana, respectively. It was prepared for two setups- one fermented by *Meyerozyma guillermondii* strain 1621 and the other by *Pichia guilliermondii* strain PAX-PAT 18S; both microorganisms were isolated from palm wine. The "must" was analysed for the proximate parameters. The starter culture was prepared by developing the inocula as described by Ogodo *et al.* [22].

2.2. Fermentation of the Wine

The fermentation of the must to wine was carried out as described by Ogodo *et al.* [22], with some modifications. The inoculum and yeast nutrients were added to the two (2) fermentation tanks and the fermentation process lasted for 28 days. At the end of the fermentation, the wines were racked with minimum exposure to air and clarified. The filtrate was allowed to age for a period of two (2) months after which, the wines were bottled and the proximate analysis of the wines was carried out.

2.3. Microbiological Analyses of the Wine

The banana and pineapple wine were allowed to ferment for twenty-eight (28) days and the viable yeast count was monitored at an interval of four (4) days. Potato dextrose agar supplemented with 50 mg/L tetracycline was used for the selective enumeration of yeast. Serial dilution of the wine was carried out and dilution of 10^{-4} was inoculated in duplicates using the spread plate method. This was incubated for three (3) days and colonies were counted [23].

2.4. Physicochemical Analyses of the Wines

Analysis of physicochemical parameters including temperature, pH, total titratable acidity, specific gravity, and the alcohol content was monitored at an interval of four (4) days.

(a) Determination of Temperature

Ten (10) ml of the "must" was put in a sterile beaker and a laboratory mercury bulb thermometer was inserted into the beaker to determine the temperature. The temperature changes in the course of the fermentation were recorded in ^{0}C.

(b) Determination of pH

Ten (10) ml of the "must" was put into a sterile beaker and a digital pH meter calibrated with standard buffers (pH 4 and 7) was inserted into the beaker to measure the pH [24].

(c) Determination of Total Titratable Acidity

The titratable acidity was determined using method 962.12 of AACI [25]. Two hundred (200) ml of distilled water was introduced into a sterile 500 ml conical flask and boiled. One (1) ml of 1% aqueous alcoholic phenolphthalein indicator solution was put into the conical flask. This was titrated with 0.1M NaOH solution to give a faint pink colour. Five (5) ml of the "must" was introduced into the boiling neutralized solution and titrated again to the endpoint using the same 0.1M NaOH solution. The titratable acidity was expressed as tartaric acid and calculated as:

$$\text{Tartaric acid} = \frac{\text{Volume of alkali} \times \text{Normality of alkali} \times 7.5}{\text{Volume of "must"}} \qquad (1)$$

(d) Determination of Specific Gravity

Fifty (50) ml specific gravity bottle was cleaned with distilled water, oven dried and allowed to cool. The weight of the cooled dried bottle was recorded as W_1. The dried bottle was filled with deionized water while the surface of the bottle was cleaned with cotton wool and weighed as W_2. The bottle was emptied and cleaned twice with 10 ml of the "must" and thereafter the bottle was filled to the brim with the "must". The surface of the bottle was cleaned with cotton wool and weighed as W_3 [26]. The specific gravity was calculated as:

$$\text{Specific gravity S.G} = \frac{S}{W} \qquad (2)$$

Where S= Weight of the volume of the "must" (W_3-W_1), and W=Weight of the volume of water (W_2-W_1).

(e) Determination of Alcohol Content (%)

The alcohol content of the wine was determined using the specific gravity of the "must" and it was calculated as:

$$\%\text{ABV} = \text{Original SG} - \text{Final SG} \times 131.25 \qquad (3)$$

Where ABV is alcohol by volume, Original SG is original specific gravity of the "must", and Final SG is final specific gravity of the "must".

3.0. Results

Table 1 presents the yeast count (sfu/ml) during the fermentation of the wines. The fermentation of the wine was monitored and controlled at an interval of four (4) days and after aging, for two (2) months. The yeast count (sfu/ml) during the fermentation of the wines was determined. There was an increase in the yeast count from day 1 to 17 and a decrease from day 17 to 85. The t-test revealed that there was no significant difference between the yeast count for both yeasts ($P \leq 0.05$).

Table 1. Mean Yeast Count (sfu/ml) During the Fermentation and After Aging of the Wines

Day	*Meyerozyma guilliermondii*	*Pichia guilliermondii*
1	6.7×10^7	5.1×10^7
5	9.3×10^7	8.4×10^7
9	1.0×10^8	1.0×10^8
13	1.5×10^8	1.3×10^8
17	1.8×10^8	1.7×10^8
21	6.0×10^7	6.5×10^7
25	1.3×10^7	1.4×10^7
85	0	0

STATISTICALLY, T-test revealed that there was no significant difference between the yeast count for the two isolates ($P \leq 0.05$).

Tables 2 and 3 present the physicochemical analysis of the wines during fermentation by *Meyerozyma guilliermondii* and *Pichia guilliermondii*. The temperature, pH, total titratable acidity, specific gravity, and alcohol content were monitored and controlled at an interval of four (4) days and after aging for two (2) months. A gradual decrease in the pH for both wines resulted in a gradual increase in the total titratable acidity. There was also a gradual decrease in the specific gravity of both wines, leading to a gradual increase in the quantity of alcohol produced. Statistically, there was no significant difference in the temperature, pH, total titratable acidity but there was a significant difference in the specific gravity and alcohol produced ($P \leq 0.05$) for both wines during and after fermentation.

Table 2. Mean Physicochemical Analyses in Wine Fermentation and Aging by *Meyerozyma guilliermondii*

Day	Temperature (^{O}C)	pH	Total titratable acidity (g/L)	Specific gravity	Alcohol production (%)
1	17.00	4.40	0.73	1.08	0.00
5	27.00	4.40	0.75	1.07	2.24
9	27.00	4.40	0.77	1.06	3.10
13	25.00	3.90	0.78	1.06	3.20
17	26.50	3.80	1.00	1.06	3.56
21	24.00	3.70	1.20	1.06	3.58
25	25.00	3.40	1.35	1.05	4.37
85	26.00	3.40	1.46	1.04	5.50

Table 3. Mean Physicochemical Analyses During the Wine Fermentation and Aging by *Pichia guilliermondii*

Day	Temperature (^{O}C)	pH	Total titratable acidity (g/L)	Specific gravity	Alcohol production (%)
1	17.00	4.30	0.74	1.08	0.00
5	27.00	4.00	0.75	1.07	2.00
9	27.00	4.00	0.77	1.07	2.20
13	24.50	3.90	0.78	1.06	2.45
17	27.00	3.70	1.20	1.06	2.51
21	25.00	3.60	1.22	1.06	3.66
25	25.00	3.40	1.35	1.05	4.36
85	26.00	3.30	1.46	1.04	6.07

4.0. Discussion

4.1. Microbiological Analyses

The result of the microbial analysis revealed that there was no contamination of the wine. This indicates that the wine was produced under aseptic conditions and it is safe for human consumption. Fermentation was carried out under aseptic conditions in order to obtain a good fermentation yield and establish the stability of the whole fermentation process throughout the period. These precautions may have been the reason why there was no contaminant and this is similar to the report in a previous study [27]. There was a gradual increase in the yeast count from 6.7×10^7 sfu/ml to 1.8×10^8 sfu/ml for *Meyerozyma guilliermondii* and from 5.1×10^7 sfu/ml to 1.7×10^8 sfu/ml for *Pichia guiliermondii* from day 1 to 17 of the fermentation. This increase in the number of yeast cells can be attributed to the effective utilization of the sugar in the "must" which led to cell propagation and multiplication. This agrees with previous findings [28] and [29].

There was a decrease in the yeast count from 1.8×10^8 sfu/ml to 1.3×10^7 sfu/ml for *Meyerozyma guilliermondii* and from 1.7×10^8 sfu/ml to 1.4×10^7 sfu/ml for *Pichia guiliermondii* from day 17 to 25 of the fermentation. This gradual decline in the yeast count from day 17 can be attributed to the decline in the sugar content in the "must" as a result of the rapid and effective utilization of the sugar by the yeast cells which in turn led to an increase in the alcohol content which will also affect the rate of yeast growth. This was in accordance with the findings of [30] and [29]. After racking, clarification and aging of the wine, there was no yeast cell present in the wine produced. This was in accordance with the findings obtained by [29].

(a) Physicochemical Analyses

The study revealed that there were fluctuations in temperature from 17 to 27 ^{0}C during the period of fermentation. This could be a result of the biochemical changes occurring during the metabolism of the substrate by the fermenting organism. The temperature fell within the range of 17 to 27^{0}C for both wines and this was in accordance with the findings of [31] that recorded a temperature of 29 ^{0}C. Obaedo *et al.* [10] also recorded the same findings with a temperature of 26 ^{0}C. This study revealed that there was a continuous decrease in the pH values in the wines during the course of fermentation. There exists a correlation between pH and the total titratable acidity of the wine. The higher the pH, the lower the total titratable acidity, and the lower the pH, the higher the total titratable acidity. This is attributable to the acidification of the medium during fermentation.

Studies have shown that during fermentation, low pH and high acidity are inhibitory to the growth of spoilage organisms but create a conducive environment for the growth of desirable microorganisms. This gives the fermenting yeasts a competitive advantage in natural environments [32]. This investigation revealed that the wine had low pH values which reduced from 4.0 to 3.4 for the wine fermented by *Meyerozyma guilliermondii* and 4.0 to 3.5 for the wine fermented by *Pichia guilliermondii.* There was a gradual increase in the total titratable acidity from 0.73% to 1.46% for the wine fermented by *Meyerozyma gulliermondii* and 0.74% to 1.43% for the wine fermented by *Pichia guilliermondii*. This was in accordance with the findings of [31] who reported a decline in pH from of 4.8 to 3.0. This similar observation was stated by [30] who recorded a decline in pH from 4.4 to 3.1 in their work and that of Obaedo *et al.* [10] also recorded a decline in pH of from 5.0 to 4.0.

There was a decrease in the specific gravity from 1.08 to 1.04 for both wines. This was in accordance with the findings of [10] that recorded a decrease in the specific gravity from 1.05 to 1.00 in their work. This led to an increase in the percentage of alcohol produced from 0 to 5.5% in the wine fermented by *Meyerozyma guilliermondii*, and 0 to 6.1% in the wine fermented by *Pichia guilliermondii*.

5.0. Conclusion

Bananas and pineapples are valuable and highly nutritious fruits, which can be fermented to produce acceptable wine. Good quality wine was produced from ripe banana and pineapple pulp using *Meyerozyma gulliermondii* and *Pichia guilliermondii*. The different parameters analyzed in this study will help to produce good quality wine and the quality of the wine can be improved during large-scale production. No chemical preservative was added to the wine, as such, further research is required to ascertain the shelf life of this wine.

Declarations

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Competing Interests Statement

Authors have declared no competing interests.

Consent for publication

The authors declare that they consented to the publication of this research work.

Authors' Contributions

All authors equally contributed to research and paper drafting.

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